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## CLINICAL STUDY

# Does having a twin brother make for a bigger brain?

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## Abstract

**Objective:** Brain volume of boys is larger than that of girls by ~10%. Prenatal exposure to testosterone has been suggested in the masculinization of the brain. For example, in litter-bearing mammals intrauterine position increases prenatal testosterone exposure through adjacent male fetuses, resulting in masculinization of brain morphology.

**Design:** The influence of intrauterine presence of a male co-twin on masculinization of human brain volume was studied in 9-year old twins.

**Methods:** Magnetic resonance imaging brain scans, current testosterone, and estradiol levels were acquired from four groups of dizygotic (DZ) twins: boys from same-sex twin-pairs (SSM), boys from opposite-sex twin-pairs (OSM), girls from opposite-sex twin-pairs (OSF), and girls from same-sex twin-pairs (SSF;  $n = 119$  individuals). Data on total brain, cerebellum, gray and white matter volumes were examined.

**Results:** Irrespective of their own sex, children with a male co-twin as compared to children with a female co-twin had larger total brain (+2.5%) and cerebellum (+5.5%) volumes. SSM, purportedly exposed to the highest prenatal testosterone levels, were found to have the largest volumes, followed by OSM, OSF and SSF children. Birth weight partly explained the effect on brain volumes. Current testosterone and estradiol levels did not account for the volumetric brain differences. However, the effects observed in children did not replicate in adult twins.

**Conclusions:** Our study indicates that sharing the uterus with a DZ twin brother increases total brain volume in 9-year olds. The effect may be transient and limited to a critical period in childhood.

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## Introduction

In the human brain, sex differences have been well studied in adults, children, and adolescents. The most consistent finding is ~10% larger total brain volume in males than in females (1–3). This volumetric difference is already present in neonates (4) and cannot be accounted for by differences in height (5). Sexually dimorphic brain areas are thought to develop under the (early) influence of sex steroid exposure (6). Evidence from animal studies suggests that prenatal exposure to testosterone (or estrogen, converted from testosterone via the aromatase-enzyme (7) leads to ‘masculinization’ (8, 9) or ‘defeminization’ (10) of the brain, whereas preventing the exposure to testosterone leads to feminization of the brain (11).

Recently, our group demonstrated that in healthy children, sex differences in brain volumes could not be explained by either pubertal testosterone or estradiol levels (12) and it was argued that prenatal exposure to sex steroids would have more pronounced effects on

(the development of) sex differences in overall human brain size than the exposure to pubertal increases of steroid production.

Studies in litter-bearing mammals have shown that intrauterine position affects naturally occurring variations in sex hormones, which are not genetic in origin (13). More specifically, a male fetus has a higher blood level of testosterone than a female fetus, but irrespective of its own sex, a fetus located between male fetuses has a higher concentration of testosterone than a fetus positioned between females (14). This phenomenon in turn influences several anatomical and behavioral parameters, such as reproductive organs and aggressive behavior (13).

In humans, the comparison of opposite-sex with same-sex dizygotic (DZ) twin pairs allows for exploration of masculinizing effects of prenatal testosterone exposure (15, 16). In human fetuses, hormone transfer may occur through two routes, i.e. the maternal–fetal transfer route (via maternal bloodstream), and the fetoplacental transfer route (hormones diffusing through

amniotic membranes) (16). Some studies applying this ‘same sex–opposite sex paradigm’ confirm the masculinizing effect of a male co-twin on a female for less left hemispheric dominance in processing verbal stimuli (17), more aggression (18), less reproductive fitness (19), and disordered eating (20). However, other studies did not demonstrate a masculinizing effect of a male co-twin e.g. for birth weight (21), aggression (22, 23), disordered eating (24), pubertal development, and fertility (25) or handedness (26). Importantly, pubertal stage and circulating testosterone levels could not account for the differences in aggression between the group having a male co-twin versus the group having a female co-twin (18). The masculinizing pattern for disordered eating was also observed in males with a male co-twin as compared with a female co-twin and socialization from growing up with a male sibling did not account for the differences in disordered eating (20).

The aim of the present study was to explore the effect of intrauterine presence of a male co-twin on masculinization of global brain volumes. Brain volumes of boys from DZ same sex twin-pairs (SSM), boys from opposite-sex twin-pairs (OSM), girls from opposite-sex twin-pairs (OSF), and girls from DZ same-sex twin pairs (SSF) were compared in order to explore whether brain volume differed by expected level of prenatal testosterone exposure. It was hypothesized that, based on their higher expected level of prenatal testosterone exposure, having a male co-twin would induce an enlargement of global brain volumes as opposed to having a female co-twin (on top of larger brain volumes in boys).

Subjects and methods

Participants

The sample was drawn from a cohort of twin pairs in which magnetic resonance imaging (MRI) scans were acquired at the age of nine years as described elsewhere (27, 28). The present sample consists of DZ twins,

including: 43 SSM children (22 pairs, 1 incomplete), 16 OSM individuals, 19 OSF individuals (coming from 19 pairs, three incomplete), and 41 SSF (21 pairs, one incomplete), with a total number of 119 children (mean age was 9.2 years (Table 1)). Exclusion criteria consisted any major medical or psychiatric illness and participation in special education. Physical health and mental health were assessed with a medical history inventory. Zygosity of the twins was determined based on DNA polymorphisms, using 8–11 highly polymorphic di-, tri- and tetra-nucleotide genetic markers. Parents and the participants themselves gave written informed consent to participate in the study. The study was approved by the Central Committee on Research involving Human Subjects (CCMO) of The Netherlands and was in agreement with the Declaration of Helsinki (Edinburgh amendments).

MRI acquisition and processing

Structural MRI scans of the whole brain were made on a 1.5 T Achieva scanner (Philips, Best, The Netherlands).

A three-dimensional T1-weighted coronal spoiled-gradient echo scan of the whole head (256×256 matrix, TE=4.6 ms, TR=30 ms, flip angle=30°, 160–180 contiguous slices; 1×1×1.2 mm<sup>3</sup> voxels, field-of-view=256 mm/70%) was acquired. Furthermore, a single-shot echo planar imaging scan was made as part of a diffusion tensor imaging (DTI)-series (SENSE factor 2.5; flip angle 90°; 60 transverse slices of 2.5 mm; no gap; 128×96 acquisition matrix; FOV 240 mm; TE=78 ms) together with a magnetization transfer imaging (MTI) scan (60 transverse slices of 2.5 mm; no gap; 128×96 acquisition matrix; FOV 240 mm; flip angle 8°; TE=4.5 ms; TR=37.5 ms), which were used for segmentation of the intracranial volume. Our imaging protocol made use of T2-weighted contrast of the DTI-B0 and MTI-series for segmentation of the intracranial volume. The intracranial volume segment was subsequently superimposed onto the T1-weighted

Table 1 Demographics of the sample.

	SSM	OSM	OSF	SSF
n (individuals)	43	16	19	41
Birth order (1st/2nd)	21/22	8/8	10/9	21/20
Handedness (R/NR)	36/7	13/3	15/4	36/5
Age (years; s.d.)	9.20 (0.11)	9.24 (0.13)	9.22 (0.14)	9.24 (0.08)
Gestational age (weeks; s.d.)	36.8 (1.7)	37.0 (2.2)	37.2 (2.0)	36.7 (1.4)
Birth weight (g; s.d.)	2739.1 (531.7)	2642.5 (551.6)	2780.3 (531.9)	2493.7 (434.7)
Testosterone (pmol/l; s.d.)*	30.8 (22.1)	23.9 (8.7)	30.9 (16.5)	38.6 (21.8)
Estradiol (pmol/l)/creatinine (pmol/l; s.d.)	115.9 (88.0)	120.0 (119.5)	119.3 (88.9)	94.9 (39.3) <sup>a</sup>
IQ (s.d.)	101.0 (12.6)	103.4 (14.5)	102.5 (10.1)	106.7 (12.1)
Height (cm; s.d.)	138.7 (5.5)	140.9 (4.8)	140.7 (5.2)	138.9 (4.8)
Weight (kg; s.d.)	31.2 (4.7)	32.3 (3.2)	32.0 (4.4)	31.8 (4.8)

SSM, males from same sex pairs; OSM, males from opposite sex pairs; OSF, females from opposite sex pairs; SSF, females from same sex pairs. Handedness: R, right handed; NR, non right-handed. Testosterone levels were available in 29 SSM, 9 OSM, 12 OSF, and 28 SSF children. <sup>a</sup>Cohen's D effect size is 0.10 (small): estradiol levels are not significantly different between the groups. Full scale IQ was measured using the Wechsler Intelligence Scale for Children (3rd Edition).

image to remove non-brain tissue voxels, as described previously (27, 29).

The scans were coded to ensure blindness for subject and zygosity identification. The T1-weighted images were automatically put into Talairach orientation (30) without scaling, by registering them to a model brain in Talairach orientation. The translation and rotation parameters of this registration were then applied to the images (31). After registration to the T1-weighted image, the intracranial segment served as a mask for all further segmentation steps. The T1-weighted images were corrected for field inhomogeneities using the N3 algorithm (32). Our automatic image processing pipeline was used for the segmentation of total brain, gray and white matter of the cerebrum and cerebellum. The software included histogram analysis, mathematical morphology operations, and anatomical knowledge based rules to connect all voxels of interest, as was validated before (33). The total brain and cerebellum segments were all visually checked and edited if necessary. Ten brains from the cohort were randomly selected and analyzed by two independent raters to estimate inter-rater reliability. Intra-class correlation coefficients were all above 0.97.

Owing to motion artifacts, separation of gray and white matter tissue was not possible in eight subjects (2 SSM, 2 OSM and 4 SSF). These subjects were included in the analyses of the total brain and cerebellum only. Consequently, the total number of individuals included in global gray and white matter analyses was 111, whereas for total brain and cerebellum volumes the total number of participants was 119.

### Hormonal measurements

Free testosterone levels were determined in first morning saliva (Competitive immunoassay (luminiscence), IBL Hamburg). The intra-assay and inter-assay coefficients of variation (CV) were below 12% at levels  $>11$  pmol/l (lower limit of detection). Total estradiol levels as well as creatinine levels were determined in the first morning urine (Competitive immunoassay (luminiscence), Architect, Abbott Laboratories). The intra-assay and inter-assay CV were 5 and 10% respectively at levels  $>150$  pmol/l (lower) and  $<9000$  pmol/l (upper). Urinary estradiol levels were divided by creatinine level to correct for variations in urine excretion rate. Both testosterone and estradiol data were collected on two consecutive days at consistent times directly after waking up, and the means of the two measurements were used in further analyses. Analyses were carried out by the endocrinological laboratory of clinical chemistry of the VU Medical Center in Amsterdam, The Netherlands. Measurable testosterone levels were available in 67% of the females ( $n=40$ ; 12 OSF and 28 SSF), and in 64% of the males ( $n=38$ ; 9 OSM and 29 SSM). Estradiol samples were measurable in all children.

### Statistical analysis

A linear regression analysis was carried out to estimate the effect of co-twins' sex (dummy-coded as: 0=girl, 1=boy) on brain volumes, corrected for the twin's own sex (dummy-coded as: 0=girl, 1=boy). More specifically, the effect of co-twins' sex was analyzed on the residuals after the effect of twin's own sex was regressed out.

Regression components and Cohen's *D* effect sizes were estimated using the software package Mx (34) that takes the dependency of the twin data into account. Birth order was taken into account by allowing mean brain volumes of the first and second born twin to be different.

Likelihood-ratio  $\chi^2$ -tests were performed to test for significance of the effect of co-twins' sex. To investigate a possible mediating role of present testosterone and estradiol levels, height or birth weight, in additional analyses these variables were subsequently included as covariates. Interactions between the twin's own and co-twins' sex were investigated as well in all analyses.

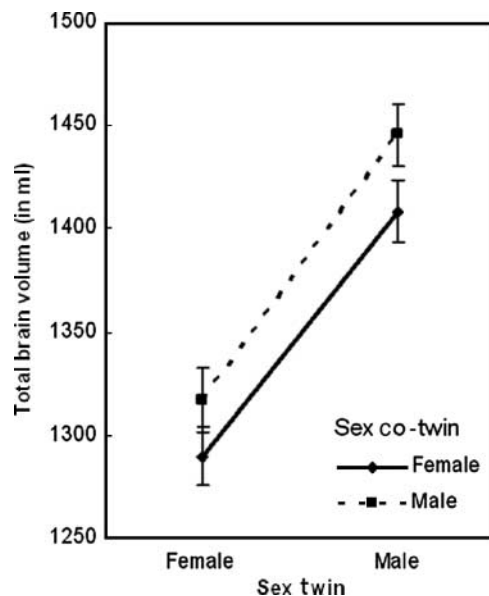
### Results

A Kolmogorov–Smirnov test showed that all brain volumes were normally distributed. When comparing the four groups of twins, no differences in age, birth weight, gestational age, handedness, testosterone or estradiol level, weight or height were found (Table 1).

### Global brain volumes

A main effect of the twins' own sex was found on global brain measures, i.e. a larger total brain ( $\chi^2=57.9$ ;  $P<0.0001$ ), cerebellum ( $\chi^2=38.7$ ;  $P<0.0001$ ), total cerebral gray ( $\chi^2=48.5$ ;  $P<0.0001$ ) and white matter ( $\chi^2=24.0$ ;  $P<0.0001$ ) in males, with a mean increase of 8.5% in male brain volumes. More importantly, results indicated that in both boys and girls, having a male co-twin is related to an increase in brain volumes (compared with having a female co-twin): a significant effect of co-twins' sex was found for total brain volume ( $\chi^2=4.22$ ;  $P<0.04$ ; (Fig. 1 and Table 2), cerebellum ( $\chi^2=3.89$ ;  $P<0.05$ ) together with a trend for total white matter volume ( $\chi^2=3.68$ ;  $P<0.06$ ). When examining the means of total brain volume between the four groups of twins, the following pattern could be observed: SSM had the largest total brain volume, followed by OSM, OSF, and SSF (Fig. 2). An absolute increase in total brain volume of 27.4 ml could be observed in OSF compared with SSF (+2%), and a 37.3 ml increase in SSM compared with OSM (+3%). The same pattern could be observed for cerebellar volumes, and although not significant, for gray and white matter volumes (Table 2). There was no significant effect of co-twins' sex on gray matter volume





**Figure 1** Effect of co-twins' sex on total brain volume. Mean total brain volume (with s.e.m.) in male or female sex with a male co-twin (dashed line) or female co-twin (solid line). Both the main effect of the twins' own sex ( $P < 0.0001$ ) and the main effect of co-twins' sex were significant ( $P < 0.04$ ).

( $P = 0.33$ ). Furthermore, no significant interaction between twins' own sex and co-twins' sex was found for total brain, gray or white matter volumes. However, for cerebellar volume, there was a significant interaction between the twins' own sex and co-twins' sex ( $\chi^2 = 9.39$ ;  $P < 0.009$ ); data indicated that a male co-twin had a more pronounced (enlarging) effect on a males' cerebellum than on a females' (+9.4 ml, (6%) vs +3.0 ml (2%)).

When correcting the analyses for testosterone and estradiol levels or height, the effects on total brain volume, white matter, and cerebellar volume remained significant. Although the effect size remained moderate, after correction for birth weight the enlarging effect of a co-twin brother on total brain, cerebellum, and white matter volumes no longer reached statistical significance (Table 2).

## Discussion

To our knowledge, this is the first study that addresses the relation between the intrauterine presence of a male co-twin on masculinization of human brain volume. Boys had significantly larger total brain, cerebellum, and gray and white matter volumes than girls, irrespective of their co-twins' sex. Confirming our hypothesis, children with a male co-twin had larger total brain, cerebellum, and white matter volumes than children with a female co-twin, on top of the overall larger brain volumes in boys. SSM had the largest volumes, followed by OSM, OSF, and finally SSF. Although the size-effect remained moderate, after correcting the analyses for birth weight, the enlarging effect of a co-twin brother on total brain, cerebellum, and white matter volumes did no longer reach statistical significance. Present testosterone or estradiol level or height did not account for the volumetric differences in children with a male or female co-twin.

The larger brain volume in boys versus girls is in agreement with earlier studies on sex differences in global brain volumes throughout development (1–5, 35, 36). Conform our expectations, having shared the uterus with a brother as compared with a sister was related to a larger brain volume. Animal research suggests that the intrauterine presence of a male fetus leads to exposure to higher levels of prenatal testosterone within other fetuses, compared with the intrauterine presence of a female fetus, leading to more masculine brain morphology (14). Furthermore, prenatal exposure to sex steroids is implicated in the development of sex differences in brain structure (8). Consequently, our findings might suggest that larger brain volumes in children with a male co-twin have resulted from higher prenatal testosterone exposure.

In animals, it has been reported that prenatal testosterone-treatment increased head circumference (37). Prenatal testosterone exposure, by influencing neuronal properties such as dendritic branching and synaptogenesis, could ultimately be reflected by an enlarged global brain volume. A probable mechanism of sexual differentiation by gonadal steroids could be apoptosis (cell death); androgens or their estrogenic

**Table 2** Global brain volumes (in ml) across same sex and opposite sex twins.

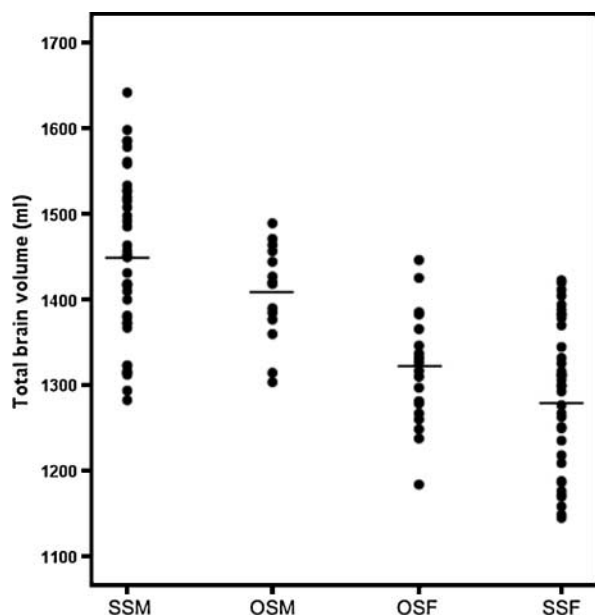
Volume	SSM	OSM	OSF	SSF	B co-twins' sex (95% CI)	D
Total brain (s.d.) <sup>a</sup>	1445.7 (95.7)	1408.3 (56.7)	1317.2 (66.2)	1289.7 (85.4)	33.5 (3.9–63.1)*24.4 (–6.7–54.9)	0.37/0.29
Cerebellum (s.d.) <sup>a,b</sup>	164.6 (10.9)	155.0 (16.7)	148.6 (11.2)	145.6 (11.0)	7.6 (0.5–14.9)*5.4 (–7.6–18.2)	0.46/0.40
Gray matter (s.d.) <sup>c</sup>	788.3 (55.0)	782.6 (36.5)	718.5 (45.0)	704.3 (54.7)	9.9 (–9.2–29.1)/6.2 (–14.2–26.7)	0.19/0.12
White matter (s.d.) <sup>c</sup>	481.6 (48.9)	460.5 (25.4)	440.1 (26.1)	429.7 (40.2)	15.5 (0.5–30.6)*10.5 (–5.1–26.1)	0.39/0.31

\* $P < 0.05$  (Significant effect of co-twins' sex). SSM, males from same sex pairs; OSM, males from opposite sex pairs; OSF, females from opposite sex pairs; SSF, females from same sex pairs. 'B co-twins' sex' represents the increase in brain volume (ml) in children with a brother compared with children with a sister corrected for twins' own sex (left of slash) and corrected for twins' own sex and birth weight (right of slash). The corresponding 95% CI are presented between brackets. Similarly, the right most column represents Cohen's  $D$  effect sizes.

<sup>a</sup>Total brain and cerebellum volumes were available in 43 SSM, 16 OSM, 19 OSF, and 41 SSF children.

<sup>b</sup>For cerebellum volume, the interaction effect between twins' own sex and co-twins' sex was included as a covariate.

<sup>c</sup>Gray and white matter volumes were available in 41 SSM, 14 OSM, 19 OSF, and 37 SSF children.



**Figure 2** Total brain volume across the four groups of twins ( $n=119$  individuals). Individuals' total brain volume (with means) across same sex males (SSM), opposite sex males (OSM), opposite sex females (OSF), and same sex females (SSF).

metabolites can either prevent or induce apoptosis (38). Interestingly, in humans, in a study on cross-sex hormone administration to transsexuals, reported that androgen treatment in female-to-male subjects was capable of increasing total brain volume towards male proportions, whereas anti-androgen and estrogen treatment in male-to-female subjects decreased total brain volumes towards female proportions (39). These data support the hypothesis that in humans, the exposure to large amounts of testosterone, similar to the prenatal surge, enlarges (masculinizes) total brain volume. After controlling for present testosterone or estradiol level, the masculinizing effects on global brain volumes remained unchanged. This indicates that (relatively low) levels of sex steroids at the age of nine cannot explain the global larger brain volumes in males, or in children with a male co-twin.

Our results are comparable with the earlier described masculinizing effects of a male co-twin on cerebral asymmetry (left hemispheric dominance in processing verbal stimuli) (17) aggression (18) and disordered eating (20). Most studies applying the 'same sex-opposite sex' twin paradigm focused on the masculinizing effects of a male co-twin on females only. However, our study suggests that an additional male in uterus might have a cumulative effect on prenatal testosterone exposure on a male fetus as well. Indeed, in rats it was found that male fetuses located between other males displayed a larger (more masculine) sexually dimorphic nucleus of the pre-optic area than male fetuses located between females (40).

We were not able to directly measure hormonal levels and brain volumes during the prenatal or neonatal period (i.e. our measurements took place at ~9 years of age).

After correcting the analyses for birth weight, the enlarging effect on brain volumes due to a co-twin brother compared to a co-twin sister did no longer reach statistical significance, although the effect sizes still remained moderate. SSF twins seem to carry this effect, as their birth weight appears lower than the rest of the sample (although not significantly different from the other groups). It might be argued that birth weight that highly correlates with neonatal head circumference (41) and most likely also with neonatal intracranial volume, is (at least) in part related to a larger brain volume at 9 years of age (which is reflected by a significant correlation of 0.28). This finding also indicates that in children with male co-twin versus a female co-twin intrauterine growth in general is increased. Indeed, in animals it has been reported that fetal growth can be programmed by prenatal exposure to sex steroids (42). On the other hand, as in our sample birth weight only explained 8% of the variance in total brain volume at 9 years of age, the use of birth weight as a proxy for prenatal testosterone exposure on brain volumes seems tenuous.

It must be noted that data on birth weight deviate from the large population based East Flanders Twin Study (EFTS), in which OSM twins were born later and reported heavier than SSM twins (43). Also, birth weights reported in the EFTS were generally lower than birth weights in our study. These deviations between both studies might be due to a selection bias in our (relatively small) sample. However, our data on birth weight and gestational age are comparable with a large data set of 2930 twin pairs from The Netherlands (44). Importantly, there was no significant difference between the educational level of mothers of twins who did and did not participate in our study (45). Furthermore, our sample forms an adequate representation of the general population with respect to general intelligence: the average IQ was 103.2 (s.d. 12.4; range 78–143) and there were no significant IQ differences between the four groups of twins or between the sexes. It is therefore unlikely that, for example, boys with higher IQs were overrepresented in our study and carried the masculinizing effect on brain volumes.

An explanation for the overall difference in birth weight between our study and the EFTS study could be that within the EFTS-study, included twin pairs were born between 1964 and 2002, whereas in our study, twin pairs were born in 1995 or 1996. It has been reported that between 1962 and 1992, birth weight in general increased significantly due to improved maternal health and less obstetric complications (46). Also it has been found recently that twins from the EFTS study (from younger cohorts) have lower birth weights than Dutch twins (M Gielen, CE van Beijsterveldt, C Derom,

**Table 3** Brain volumes of adult dizygotic twins ( $n=116$ ).

	SSM	OSM	OSF	SSF
<i>n</i> (individuals)	44	11	21	40
Age (s.d.)	29.7 (8.3)	30.5 (13.5)	29.9 (12.7)	30.1 (8.4)
Total brain (s.d.)	1301.9 (84.9)	1299.2 (87.6)	1171.7 (86.2)	1180.5 (110.9)
Gray matter (s.d.)	767.8 (62.3)	791.3 (50.5)	724.6 (71.9)	725.0 (63.5)
White matter (s.d.)	534.1 (45.5)	507.9 (53.2)	447.1 (42.7)	455.5 (60.8)
Cerebellum (s.d.)	143.3 (9.6)	143.9 (12.3)	132.7 (11.3)	135.0 (14.0)

SSM, males from same sex pairs; OSM, males from opposite sex pairs; OSF, females from opposite sex pairs; SSF, females from same sex pairs. Mean (s.d.) age in years, mean (s.d.) brain volumes in ml.

R Vlietinck, JG Nijhuis, M Zeegers & DI Boomsma, personal communication).

We can only speculate, whether the enlarged brain volumes in children with a male co-twin are indeed a result of prenatal factors or in fact of postnatal factors, such as play-or socializing behavior. However, it seems unlikely that having shared the social environment with an opposite sex co-twin would have a substantial influence on neuroanatomical phenotypes. In a post-hoc analysis on part of the sample, we explored the possible effect of an older male or female singleton sibling (corrected for twins' own sex):  $n=37$  girls: 18 with an elder sister, 19 with an elder brother;  $n=37$  boys: 22 with an elder sister, 15 with an elder brother, all from same sex twin-pairs. Results of this post-hoc analysis showed no differences in brain volumes of children with an older singleton sister compared with children with an older singleton brother. These data support a role for prenatal rather than post-natal effects on masculinization of brain volumes. Of course, this post-hoc analysis requires the assumption that the influence of an older singleton brother or sister is the same as for a twin brother or sister. The unique influence of the presence of a – opposite sex – co-twin during childhood is obviously present and its influence cannot be excluded in this analysis. In addition, in an attempt to replicate our observations, we investigated another dataset of brain volumetric MRI measurements in twins we had access to (47, 48). However, in that sample with a mean age of 30.0 years ( $\pm 9.7$  years), no enlarged brain volumes in subjects with a co-twin brother were observed compared with subjects with a co-twin sister (Table 3). It might be argued that possible prenatal masculinizing factors on human brain volume are transient and limited to a critical period in childhood, since the effect could not be demonstrated in adults. The absence of effect during adulthood could also suggest that both organizational (prenatal) and activational (resulting from circulating post-pubertal levels of hormones) effects of sex steroids are important for brain volume. Thus, it may be argued that before puberty, prenatal testosterone influences brain volume (which may have behavioral effects), but when hormones are activated at puberty, they may override the prenatal effect on brain volume (12, 39).

We cannot state that the mechanism underlying prenatal masculinizing of brain volume in children is a

specific result of testosterone, or might in fact result from other hormonal exposure. One such hormone is 17 $\beta$ -estradiol, an important metabolite of testosterone which is converted through the aromatase enzyme in both sexes. For example, it is plausible that a higher rate of conversion into estradiol in females is responsible for 'demasculinization' or 'feminization' of brain volume, although evidence for this view is limited (6). Moreover, there appears to be a dramatic sex difference in prenatal testosterone level in the human fetus between week 8 and 24 of gestation, a critical period of brain development (49). Prenatal estrogen levels do not show such a remarkable sex difference, making it likely that during critical periods of brain development, testosterone is implicated in producing sexual dimorphisms. In humans, it is at present unclear how a possible increased prenatal testosterone exposure is caused. Although it has been reported that next to the direct feto–fetal route, hormone transfer in humans may occur through the indirect maternal–fetal transfer route (via maternal bloodstream) (16), it has more recently been found that maternal serum steroid levels are unrelated to fetal sex of twins (50). This would make the direct feto–fetal route more plausible as a source of prenatal testosterone exposure. Furthermore, it should be noted that sex steroids are also produced within the placenta (51). Thus, the effect of the co-twin is not due to hormones from the co-twin, but might rather reflect general growth patterns in boys and girls due to placental characteristics (52).

To conclude, our results indicate that having shared the uterus with a brother does seem to increase total brain volume compared with having shared the uterus with a sister. This effect may be related to a higher level of prenatal testosterone exposure. Future studies in larger samples including neonatal subjects are required to further investigate this issue and replicate our findings.

## Declaration of interest

Authors do not report any conflict of interest.

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